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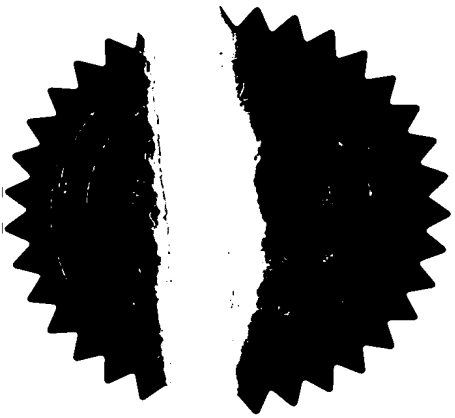

Signed R. Mahoney

Dated 29 October 1999

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Request for grant of a patent

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1. **Your reference** 44.65733/002

26 NOV 1997

2. **Patent application number** *(The Patent Office will fill in this part)* **9725007.0**

27NOV97 E320408-13 D00027
P01/7700 25.00 - 9725007.0

3. **Full name, address and postcode of the or of each applicant** *(underline all surnames)*

1) Nycomed Imaging AS
Nycoveien 1-2, N-0401 Oslo, Norway
2) The General Hospital Corporation
55 Fruit Street, Boston, Massachusetts
02115, USA

Patents ADP number *(if you know it)*

8609370003

If the applicant is a corporate body, give country/state of incorporation

1) Norway 2) USA

4. **Title of the invention** Method

5. **Name of your agent** *(if you have one)* Frank B. Dehn & Co.

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

179 Queen Victoria Street
London
EC4V 4EL

Patents ADP number *(if you know it)* 166001

6. **If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and** *(if you know it)* **the or each application number**

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. **If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application**

Number of earlier application	Date of filing *(day / month / year)*

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a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
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11. **I/We request the grant of a patent on the basis of this application.**

Signature Frank B Dehn & Co **Date** 26 November 1997

12. Name and daytime telephone number of person to contact in the United Kingdom

Julian Cockbain
0171 206 0600

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Method

This invention relates to improvements in and relating to methods of embolus therapy, eg. methods for the treatment of tumors, inoperable aneurysms and other vascular disorders where surgery may not be a viable option or for reducing bleeding during surgery, and to pharmaceutical compositions used in such methods.

While emboli, stoppages of blood flow, are normally considered to be undesirable and sometimes are life-threatening, embolus generating agents have been used in certain fields of medical treatment, generally to block off blood supply to tumors or to tissue which is to be operated upon surgically. In the former case, embolization, optionally combined with chemotherapy (chemoembolization), achieves a beneficial cytotoxic effect. In the latter case, blood loss is reduced and surgery is facilitated. In either case the embolus generating agent is usually administered via a catheter into an artery upstream of the site at which embolus formation is to occur.

Because embolus formation is generally undesirable, in embolus therapy it is particularly desirable that the embolus formed should be detectable by a diagnostic imaging modality (such as X-ray, MR imaging or ultrasound). However of the embolus generating agents currently in medical practice, only Lipiodol (Ethiodol) is amenable to imaging.

Lipiodol comprises droplets of an iodinated poppyseed oil and is observed by radiographic imaging to show where the embolus has localized. This approach however has the drawback that the oil droplets are susceptible to breaking up to form smaller droplets which may pass

downstream of the target embolus site and cause emboli to form in tissues remote from the target organ, eg. in the lungs. As a result significant adverse events can result from this misdirected migration of the oily agent. The embolus may lodge too proximally to the intended site, allowing collaterisation of the target bed and may also translocate after an uncertain time. Thus with Lipiodol the behaviour of the embolic material in use cannot be accurately predicted.

Other conventionally used embolus generating agents, such as gelfoam, are not themselves detectable by imaging modalities and require the administration of a conventional water-soluble contrast agent (eg. an X-ray agent such as Omnipaque or an MRI contrast agent such as Omniscan or Magnevist) to enable the location of the embolus to be determined. This may be done by tracking the blood vessel of interest to detect the point at which contrast enhancement ceases. It is assumed that the embolus is located at the point where contrast agent is blocked from further passage down the vessel of interest. This can however result in inaccurate diagnoses and diminished prognoses for the patient if the embolus is not actually located at the point where contrast enhancement stops being evident on the image.

The use as chemoembolization agents of agarose gel particles loaded with a cytotoxic agent or a soluble X-ray contrast agent has been proposed by Kishi et al. in Nippon Acta Radiologica 55: 300-304 (1995). However the quantity of contrast agent that can be loaded into the matrix of a carrier particle is limited due to the size constraints on the overall particle (the particle size is dictated by the diameter of the blood vessel in which location of the embolus is sought - too large and the particle will not reach the desired location, too small and the particle will pass downstream of the desired

location with the accompanying risk of adverse events mentioned above for Lipiodol) and the proportion of particle volume made up by the matrix material itself. Moreover detection of the embolus by imaging requires very small concentrations of embolus forming particles to be detectable and agarose gel particles of appropriate size containing X-ray contrast agents will not provide a satisfactory contrast enhancement for monitoring both initial placement of the embolus, and long term monitoring of the therapeutic application.

While radiolabels could be detected even when present at very small concentrations at an embolus, the use of radiopharmaceuticals is generally complex (eg. requiring the generation of the radiolabel shortly before administration) and is not preferred by the medical community. Furthermore, scintigraphy is not usable for monitoring the intervention in progress, i.e., determining the targeting, dosimetry etc. Moreover the embolus generating agent may remain in place for a prolonged period and in such circumstances the use of radiolabels is again not preferred.

There is thus a need for a non-radioactive embolus generating agent which is contrast effective at the concentrations achievable at the embolus, which is not prone to forming undesired emboli at locations remote from the target tissue, and which may be used to monitor placement and longer term persistence of the embolus.

It has now been found that solid water-insoluble particles of a non-radioactive diagnostically effective compound and vesicles encapsulating a non-radioactive diagnostically effective compound or a solution thereof may be used effectively as embolus generating agents and that the emboli thereby generated are detectable by diagnostic imaging modalities.

Thus viewed from one aspect the invention provides a method of embolus therapy comprising administering into the vasculature of a human or non-human animal (preferably mammalian) subject a composition comprising particles of a size or formulation selected to generate emboli at a target site within said subject, characterised in that as said particles are used solid water-insoluble particles of a non-radioactive diagnostically effective compound or vesicles encapsulating a non-radioactive diagnostically effective compound or a solution thereof, and in that embolus location is detected by a diagnostic imaging technique. The objective is to reduce perfusion or extravasation of the target region.

Viewed from a further aspect the invention also provides the use of solid water-insoluble particles of a non-radioactive diagnostically effective compound or vesicles encapsulating a non-radioactive diagnostically effective compound or a solution thereof for the manufacture of an embolus generating pharmaceutical composition for use in chemoembolus therapy.

By diagnostically effective it is meant that the compound is capable of detection by a diagnostic imaging modality, eg. X-ray, ultrasound, MRI, magnetotomography, light imaging (including near infra red imaging) or electrical impedance tomography, and thus that emboli created by the particles comprising such diagnostically effective compounds may be located and monitored by such imaging modalities. Such compounds will generally be referred to herein as contrast agents. By therapy, it is meant that therapeutic materials may be deposited, in accordance with the invention, in a precise location by embolization.

The particles used according to the invention are either particles of a solid contrast agent or are particles (vesicles) encapsulating a contrast agent which may be in solid, liquid or gas phase. In the former case, the particles may comprise a core surrounded by a coat and the solid contrast agent may make up either the core or, more preferably, the coat. Where the contrast agent forms the coat (eg. about a polymer bead) it will be water-insoluble, while where it forms the core it will be water-insoluble if the coat is porous or water soluble. With a water insoluble coat a water soluble solid contrast agent core may be used.

The precise structure adopted for the particles will to a large extent depend on the means by which the contrast agent achieves contrast enhancement in the chosen imaging modality. Thus, for example for X-ray imaging techniques, contrast enhancement is generally achieved by X-ray attenuation by heavy atoms in the contrast agent. The attenuation effect is not dependent on the chemical environment of these heavy atoms (high density materials) and accordingly the contrast agent may be on the outside or on the inside of the particle or may make up the entire particle. For T_1 dependent MRI contrast agents, contrast effect is dependent on chemical environment and accordingly the contrast agent should form the surface of the particle or should be in an aqueous environment in the core of a vesicle. For such T_1 contrast agents, the particle may advantageously be a porous particle of or containing a water-insoluble or non-leaching contrast agent.

For particulate T_2 or T_2* agents, eg. superparamagnetic metal oxide crystals, the particles may conveniently be held by a polymeric carrier, eg. of a biodegradable polymer, so that eventual biodegradation of the polymer releases the particulate contrast agent and removes the

blockage to blood flow, or in certain circumstances where a more permanent blockage is required, the polymeric carrier may be refractory or the particles alone may be sufficient to cause the embolus.

For ultrasound imaging techniques, the contrast agent should be echogenic and may suitably be a gas (or a gas precursor which generates a gas at body temperatures) enclosed within a vesicle which causes embolization at the desired site.

The contrast agent in the particles used according to the invention may for example be a water-insoluble solid iodinated organic compound, eg. a triodophenyl compound such as those described in US-A-5,318,767, US-A-5,451,393, US-A-5,352,459, US-A-5,569,448, eg. NC 8883 or NC 12901. Other X-ray contrast agent particles may be produced by coating an inert particle (eg. a glass, polymer or inorganic solid bead) with an insoluble X-ray opaque compound. This would reduce the load of contrast agent and yet provide contrast during imaging. Thus for example a suspension of inert polymer beads in a solution of a water-insoluble iodinated contrast agent in a non-aqueous solvent may be mixed with water to cause the iodinated agent to precipitate on the bead surface to produce a particle sufficiently large to be an embolus generator and sufficiently X-ray dense to be visualizable. Particle size may be controlled by the rate of water addition and by the amount of water added prior to particle recovery by filtration or centrifugation. Further suitable X-ray agents include water-insoluble iodinated liquids provided with a surface coating or crosslinked at the surface to prevent particle break up on administration.

Inert metal oxides and insoluble metal salts, (eg. sulfides and sulfates) (eg. of metals of atomic number

greater than 22) may also be used as embolus generators. Thus for example particles of insoluble metal oxides and salts are available commercially in a range of particle sizes from 0.1 mm to 1 mm and larger. Zirconium oxides, zirconium silicates, yttrium oxides and other transition metal oxides may be mentioned in this regard and may be obtained commercially. Similarly beads of inert metals such as gold or platinum may be used in this regard. These materials are X-ray dense and very inert; moreover they can readily be purified by heat depyrogenation and steam sterilization. Much smaller metal oxide particles (eg. titanium oxides) are also available, eg. from vapour deposition processes. Again these can readily be purified by the same techniques. Tungsten oxides either alone or in combination with other metals are particularly suitable due to their X-ray opacity.

A particular interesting insoluble metal salt is the phosphate salt of calcium known as hydroxyapatite. This material is the major component of bone and is porous. It is commercially available from a number of vendors and can be processed to very small particle sizes, although particle sizes in the range of tens of microns are generally desired for the present invention. This material, either suspended in water or in the presence of conventional soluble X-ray contrast agents is X-ray dense (i.e. like bone) and causes the desired embolic effect throughout the capillary bed of exposed tissues. Examples have been reported of the use of very small particles of hydroxyapatite (<200 nm) for MRI contrast in liver, spleen and blood after doping with a magnetically active metal ion like manganese or mixed ion oxides (see US-A-5560902, US-A-5419892, and US-A-5342609). Those examples are included herein by reference for the preparation of embolic particles having those same MRI activities for diagnostic MRI imaging of embolized tissues. In addition,

hydroxyapatite is expected to have advantages in drug delivery over particles of pure X-ray contrast agents inasmuch as it is porous and can be used to sequester therapeutic moieties, such as oncologics and biologics such as TNF, IL1, IL2, etc., and radioactive nuclei for interstitial radiotherapy of tumors and other lesions.

Where the contrast agent is to function as an MRI contrast agent, especially as a T_1 agent, it may be particularly advantageous to deposit the contrast agent on an inert particle (eg. of a polymer such as polystyrene or polylactic acid, or a glass or ceramic particle such as ZrO, $ZrSiO_2$, TiO_2, Al_2O_3 etc.) or in porous particle (eg. a zeolite) of the appropriate size. By way of example, mixing a low pH solution of gadolinium (III) chloride and a high pH solution of sodium oxalate with stirring in a vessel containing the carrier particle in suspension would cause precipitation of gadolinium oxalate, an MRI active solid, on or in the carrier particle.

As ultrasound embolization agents one may use vesicles (eg. liposomes, micelles or microballoons) containing an echogenic gas or gas precursor (eg. air, oxygen, nitrogen, carbon dioxide, helium, sulphur hexafluoride, low molecular weight hydrocarbons, or fluorocarbons (eg. perfluoroalkanes such as perfluorobutane or perfluoropentane)). The vesicle membrane may be for example a lipid (or mixture of lipids) or it may alternatively be a polymer. Where ultrasound destruction of the vesicles is desired, the membrane will preferably be relatively frangible, eg. as in the Cavisome product of Schering AG. The ultrasound embolizing agents will preferably be coformulated with conventional (smaller and/or more flexible) echogenic ultrasound agents (eg. gas filled vesicles) to enable embolus placement to be followed more readily.

Alternatively the suspension medium for the embolization agent may contain a surfactant and may be shaken to produce surfactant-stabilized microbubbles before administration.

Simple polymer beads, or particles of a chromophore optionally provided with a light transmitting coating, can be used according to the invention as light imaging effective embolus generating agents. Likewise, for magnetotomography magnetic particles (ie. ferro-, ferri- or superparamagnetic particles, eg. iron oxide or mixed oxide particles) may be used as detectable embolus generating agents. In this case the particles may be composite particles of a non-magnetic matrix and one or more magnetic particles and as the matrix one will preferably use a biodegradable polymer so that on degradation the magnetic particles are released and in due course taken up by the reticuloendothelial system. In instances where a more permanent embolus is desired, the polymer may be refractory to degradation or the magnetic particles may themselves be of such a size as to form the embolus without need of a matrix polymer.

In one preferred embodiment, the embolization agent according to the invention comprises particles of polyvinylalcohol (PVA) incorporating a diagnostically effective material, eg. a paramagnetic or superparamagnetic material, an iodinated X-ray contrast agent or a heavy metal compound, a radioactive material, etc. as discussed herein. For this invention, these PVA particles will preferably have a particle size below 50 μm, especially below 20 μm, so as to function as capillary embolic agents. Moreover, they may advantageously be treated so that they are highly charged or are coated with a charged coating material, eg. a surfactant. Particles incorporating paramagnetic or heavy metal ions or compounds or insoluble salts

thereof or iodinated organic compounds are particularly preferred as these may be produced in a straightforward fashion. Thus such particles can be prepared by equilibrating PVA particles in a solution of the metal ion of interest (eg. Mn, Fe, Gd, Dy, W, Ba, etc.) such that the pores and surfaces of the PVA particles act like an ion exchange resin and adsorb the metal ions of interest. This will normally be done in a low pH, aqueous solution wherein the particles swell some 20% in volume and the metal ions are soluble. After equilibration, the particles can be separated by filtration or centrifugation or any other physical method from the solution phase. This can also be done by diafiltration. The particles may then be resuspended in an elevated pH solution such that the adsorbed metal ions are converted to insoluble metal oxides thus yielding PVA particles with entrapped heavy metal particles for CT and/or MRI contrast. Alternatively, the metal ions can be precipitated with salt solutions rather than the elevated pH. For example, Mn can be precipitated by the addition of carbonate, phosphate, or silicate while Fe can be precipitated with any number of salts including analytical reagents and some iodinated contrast agents like sodium hypaque, and sodium iodipamide. Thus, PVA particles can be prepared via relatively simple solution chemistry which have either MRI or CT dense particles encapsulated within. PVA particles may likewise be produced with both MRI and CT dense agents encapsulated by using a mixture of metal ions in the initial solution equilibration.

The particle containing compositions used according to the invention will advantageously comprise a liquid (preferably aqueous) carrier medium and preferably that carrier medium will contain a dissolved or smaller particulate contrast agent, particularly preferably an agent effective for contrast enhancement in the same

imaging modality as the embolus generating particles. These dissolved or smaller contrast agents may be diagnostically effective in the same or in a different imaging modality as the larger embolus generating particles. For example iohexol may be used in conjunction with an MRI-active embolic agent or gadodiamide may be used in conjunction with an X-ray opaque embolic agent. In this way the placement of the embolus may be detected even more effectively in real time. While the extra contrast agent is preferably in solution or suspension in the carrier medium (eg. being a soluble iodinated X-ray contrast agent such as iohexol, iodixanol, iopamidol, ioversol, iotrolan, metrazamide, etc. or a soluble MRI contrast agent such as Gd DTPA, Gd DTPA-BMA, Gd DOTA, Gd HP-DO3A, Mn DPDP, etc.), particulate agents may also be used if these are smaller than the particle size necessary to generate emboli (eg. gas filled vesicles, iodinated organic compound containing vesicles, superparamagnetic particles or gadolinium oxalate particles, etc).

Furthermore, in the method of the invention a cytotoxic agent will preferably be administered before or with the embolus generating particles. Chemoembolization is an established technique and a range of suitable cytotoxic agents is known, eg. carboplatin, mitoxantrone, epirubicin, mitomycin C, decarbazine, vinblastine, cisplastin, interferon, dactinomycin, hydroxyurea, carmustine, methyl CNNU, interleukin-2, cyclophosphamide, amsacrine, doxorubicin, etc. This agent may be used at conventional cytotoxic doses (see for example Ryder et al. Gut 38: 125-128 (1996), Bedikian et al. Cancer 76: 1665-1670 (1995), Bronowicki et al. Cancer 74: 17-24 (1994) and Bartolozzi et al. Radiology 197: 812-818 (1995)). In one preferred embodiment of the invention, the particulate embolus generating agent also contains a cytotoxic agent,

preferably a poorly water soluble compound, eg. within the pores of a porous particle or as a surface coating on an insoluble contrast agent particle. In this way the cytotoxic agent is released gradually from the particle following embolus formation so as to achieve an enhanced cytotoxic effect deriving from blood flow stoppage, from the released cytotoxic agent, and from the cytotoxic agent delivered before embolus formation occurred. As an alternative to a cytotoxic agent, a radio-pharmaceutical can be used.

In a further preferred embodiment, the particulate embolus generating agent also contains an angiogenesis-inhibitor as proposed by Okada et al. in US-A-5202352.

The embolus generating agents used according to the invention will have a particle size appropriate for embolus generation in the target tissue of interest. For embolus formation in capillaries, the particle size may be in the range 5 to 20 μm, ie. much smaller than traditional embolus generating particles which generally serve to block a feeding artery for a tumor rather than the capillary vessels of the tumor itself. By blocking the capillaries using the particles according to the invention, the likelihood of collateral bypass of the intended embolization is reduced. Whilst contrast effective particles with sizes up to about 8 μm which can pass through the capillary are known as diagnostic imaging contrast agents, the small capillary blocking contrast effective particles are novel and they and pharmaceutical compositions thereof form a further aspect of the present invention. It should be noted that the larger known diagnostic agent particles with sizes above 5 μm are flexible particles which can deform to transit the capillaries - the capillary blocking particles of the present invention will on the other hand be inflexible particles when the particle size is

towards the bottom of the 5 to 20 μm range, eg. at 12 μm or below.

In general, for capillary embolization, the particle size will preferably be 7 to 15 μm, especially 8 to 12 μm.

Alternatively, smaller particles, of a size normally associated with use as diagnostic imaging contrast agents can be used as embolus forming agents if components normally added to their pharmaceutical formulations so as to prevent aggregation or gel formation are omitted or used in reduced concentrations. Thus one may use particles conventionally thought to be too small to cause emboli, as they are capable of passing through the capillary beds. However, the formulation of these agents is such that they can be prepared and sterilized and be physically and chemically stable, yet upon exposure to the biological environment of blood or tissues particles either aggregate or gel to form the desired emboli. While the emboli creation can be controlled by particle size, the formulation is also important in the formation of the emboli. For example, conventional nanoparticle surfactants such as the Pluronics may be omitted from these formulations since they retard aggregation and gel formation and hence afford a greater probability that emboli may form elsewhere in the body other than the desired site.

Thus in such formulations, particles which would be thought to be capable of passing through the capillary beds by virtue of their size will still qualify as embolus-forming contrast effective particles.

The method of the invention may also be used to block larger blood vessels, eg. the larger feeding arteries leading to the tissue site of interest (eg. a tumor or a

site intended for surgical intervention) and in this case larger embolus generating particles may be used, eg. having particle sizes up to 2 mm, preferably from 50 to 1500 μm, especially about 100 μm. Pharmaceutical compositions containing such large (> 20 μm) particles in a physiologically tolerable sterile aqueous carrier medium are also novel and form a further aspect of the invention.

Appropriate particle sizes can be achieved by size separation of polydisperse particle mixtures, by milling, or by the use of core particles of appropriate size, controlled by precipitation or crystallisation: Milling can include dry milling, jet milling, wet milling or any other particle size enhancement via attrition processes. In addition a microfluidizer can be used to disperse and prepare these particle suspensions via the shear and impact of that process. This is controllable by the number of passes (i.e., resonance time) and the applied pressure. There are a number of particle preparation procedures which can be used to control the size of the core including thermolysis of solutions or suspensions, evaporative precipitation and ultrasonic dispersion.

The embolus generating particles used according to the invention are preferably capable of being broken down in vivo, either over a prolonged period of time or relatively rapidly once the need for the embolus is removed (eg. following the surgical intervention when an embolus has been created to reduce bleeding during an operation). However permanent embolization can also be achieved with embolic agents according to the invention, eg. agents which are poorly biodegradable.

For relatively large embolus generating particles particle breakdown can be achieved by laser lithotripsy,

by guiding a light transmitting fibre to the particle and subjecting the particle or the immediately adjacent plasma to a burst of light energy. For smaller particles, alternative breakdown mechanisms are necessary. For gas containing vesicles, a high energy pulse of ultrasound may be used to burst the particles and remove the blockage to blood flow. Indeed this technique may be used downstream of the embolization site during embolus formation to destroy any embolus generating particles that are not retained at the target site and so prevent unwanted emboli from being formed elsewhere. The technique similarly may be used to enhance cytotoxic drug delivery where smaller gas and cytotoxic agent containing particles are administered to create capillary emboli followed by larger gas-free particles to cause further upstream embolization. Subsequent to location of both sets of particles, the smaller particles may be burst by ultrasound to release the cytotoxic agent in a flow free zone in or adjacent the tumor.

Alternative methods of ensuring breakdown of the embolus generating agent include the use of contrast agents or coatings (or vesicle membranes) which while effectively water-insoluble, are biodegraded, eg. due to the presence of ester bonds or other biodegradable linkages. It is also feasible that other interventional techniques can be used to remove the emboli, such as surgical resection and other removal techniques <u>viz</u> vacuum removal.

The embolus generating particles may be formulated for administration together with conventional pharmaceutical excipients or other 'active' agents, including for example: soluble or capillary transitting contrast agents (as discussed above); cytotoxic agents (as discussed above); liquid carrier media (eg. pyrogen free

water, saline, water for injections and ethanol); salts (eg. of plasma cations with physiologically tolerable counterions), sugars, sugar alcohols and other osmolality adjusting agents; viscosity modifiers, emulsifiers and stabilizers; buffers and pH adjusting agents; polyethylene glycols, etc.

It is thought that iso-osmotic preparations or slightly hyperosmotic preparations will function better than hypo-osmotic suspensions, although all appear to work well.

The particle concentration and the dosage will depend upon the patient, the selected particle size, the intended embolization location and the administration route. Since administration will generally be via injection, preferably via a catheter, upstream of the intended embolization location, the number of particles required will clearly be dependent upon the number of paths downstream of the injection site which are capable of being blocked by the particles. However particle concentrations will preferably be below 20% wt/vol in the overall compositions and more preferably below 10% and where a soluble contrast agent is included in the carrier medium this will preferably be at a concentration of less than 10% wt/vol for MRI but greater than 5% for CT and more preferably greater than 20% as soluble agents will be viewed by fluoroscopy which is less sensitive than CT and requires increased agent.

Thus by way of example, in animal experiments, 50 μL was effective for rat brain whereas 100-250 μL was effective for myocardium or kidney.

The particles of the invention may particularly suitably be used to reduce actual or anticipated blood leakage

(eg. during surgery), and in embolization and chemoembolization therapy of tumors, particularly heptatocellular carcinomas, head and neck tumors, renal tumors and other solid tumors.

The embolic agent used according to the invention preferably comprises particles already of a size appropriate to cause embolus formulation at the desired site. As an alternative however, the invention may involve administration of a composition which is reactive with body fluid (eg. blood) to produce particles of the appropriate size and composition. This represents a further aspect of the invention. In this aspect, the composition will contain a biologically compatible liquid solvent system (eg. containing an alcohol, ester, ether or DMSO) and a particle forming or particle enlarging agent which is less soluble in the body fluid than in the liquid solvent system. Such an agent may be a biologically compatible polymer which enlarges particles of an iodinated organic diagnostic agent present in the composition or which forms particles entrapping such an iodinated organic agent in solution in the liquid solvent system. Alternatively, the particle forming agent may be a diagnostically effective agent which is soluble in the liquid solvent system but forms particles or droplets on contacting body fluids such as blood.

Examples of such diagnostically effective agents include the water insoluble or poorly water soluble solid or liquid iodinated organic compounds disclosed in the patent publications of the 1990's from Sterling Winthrop Inc.

Iodinated agents which could be useful in such applications include degradable agents containing labile ester functionalities and agents which are essentially

not degradable. Additionally, agents which are oils and are soluble in the solvent system for the polymer can be used. While these oils are not particulates, they are water insoluble and inert with respect to degradation and would be captured within the precipitating polymer as the embolus is formed.

In this aspect of the invention, the composition before administration preferably is free of particles, ie. the diagnostic agent is preferably soluble in the liquid solvent system.

The benefit of this formulation is that it does not require particles per se in the dosage form thereby obviating any problems within the catheter during dosing due to aggregation, etc. This improvement also extends to the shelf stability of the embolic agent with respect to settling of the contrast gents and aggregation, resuspension, etc. Also, the organic nature of these agents may make them much more compatible with the precipitating polymers serving to bind the various polymer segments together for a more permanent blockage. Lastly, some of these agents have demonstrated excellent safety upon iv injection (as nanoparticle suspensions or oil-in-water emulsions) suggesting that they would have an advantage over materials like barium sulfate and other inorganic particles.

Patents and other publications referred to herein are hereby incorporated by reference.

The invention will now be illustrated further with reference to the following non-limiting Examples in which parts, percentages and ratios are by weight unless otherwise specified.

EXAMPLE 1

NC 12901: Ethyl (3,5-diacetamido-2,4,6-triiodobenzoyloxy)acetate (US Patent 3,097,228)

A mixture of 63.6g (0.1 mol) of sodium diatrizoate and 14.7g (0.12 mole) of ethyl chloroacetate in 175 ml of dimethylformamide was heated on a steam bath with stirring for six hours. The reaction mixture was filtered while hot and the filtrate was diluted with cold water to a volume of 500 ml. The solid material which had separated was collected by filtration and stirred with 500 ml of 5% sodium bicarbonate solution. The product was again collected by filtration, washed with water, followed by ether and then dissolved in 300 ml of hot dimethyl formamide. The resulting solution was filtered, diluted with 350 ml of hot water and cooled. The resulting product was collected by filtration and dried to give 53g of ethyl (3,5-diacetamido-2,4,6-triiodobenzoyloxy)acetate, mp 269.5-270.5°C (dec.).
Calculated for $C_{15}H_{15}I_3N_2O_6$: C 25.73; H 2.15; I 54.4;
Found: C 25.80; H 2.77; I 53.8.

EXAMPLE 2

Embolization Composition

A 20 ml slurry of NC 12901 was prepared using 2.0g of NC 12901 and 1.0g of iohexol (solid) in 18.31 ml of water. This slurry was added to a 1 oz brown glass bottle along with 15 ml of 1.1 mm diameter zirconium silicate milling beads. The resulting slurry was 10% NC 12901 and 5% iohexol (wt/vol %). This slurry was rolled at approx. 100 rpm overnight. At the end of that time, the slurry had been transformed into a white, milky suspension. The suspension was separated from the milling beads by

pipetting or by filtration through coarse mesh screen. Particle size was determined by light scattering using a Horiba 910a particle sizing instrument. After milling, the average particle size was determined to be 3.96 microns with a broad standard deviation of 2.56 microns. After autoclaving, the average particle size was determined to be 8.10 microns, again with a broad particle size distribution of 3.90 microns. These large particles settle slightly with time but are easily resuspended with gentle shaking.

EXAMPLE 3

Embolization Composition

A 20 mL suspension in water of 10% NC 12901 and 10% iohexol (wt/vol %) was added to a 1 oz amber wide mouth bottle containing 15 mL preconditioned 1.1 mm $ZrSiO_3$ beads such that the bottle was just full to the top. Care was taken to minimize or remove any head space from the bottle. The entire 20 ml suspension did not fit into the jar with the milling beads and some of the suspension was not milled and thus was discarded. The sample bottle was allowed to roll on a US Stoneware 3 tiered roller mill (East Palestine, Ohio) at approximately 125 rpm for 24 hours. At the end of this time, the suspension was separated from the milling beads by pipetting or by filtration through a coarse mesh screen. Particle size and pH were measured using the Horiba LA910 (Irvine, California) particle-size analyzer and a standard digital pH meter. The average particle size was 2.6 μm. Samples were diluted in 0.001% dioctyl sulfosuccinate for size measurement. The harvested suspensions were then autoclaved for 15 minutes at 121.1°C in standard crimp sealed glass vials at half fill. The particle size and pH were measured after autoclaving. The average particle size was 5.6

μm.

EXAMPLE 4

NC 8883 - Ethyl 3,5-diacetamido-2,4,6-triiodobenzoate

To 8.11 L of dry dimethylformamide was added 1.01 kg (1.65 mole) of diatrizoic acid. To the vigorously stirred suspension was carefully added 274g (1.99 mol) of milled potassium carbonate. During the addition, there was significant gas evolution. Before all of the solid had gone into solution, a second solid began to form at the end of the carbonate addition. The mixture was stirred for 30 minutes at room temperature followed by the dropwise addition of 608g (3.90 mole) of ethyl iodide. After stirring overnight, the mixture had become essentially homogeneous and was poured into 25 L of water. The resulting precipitate was collected by filtration, washed thoroughly with water, and dried under vacuum at 60°C to afford 962g (91%) of ethyl 3,5-diacetamido-2,4,6-triiodobenzoate as a white solid, mp 280-290°C (dec.).
Calculated for $C_{13}H_{13}I_3N_2O_4$: C 24.32; H 2.05; N 4.36;
Found: C 24.27; H 1.93; N 4.28.

EXAMPLE 5

Embolization Composition

Example 3 was repeated using NC 8883 in place of NC 12901. The average particle size before autoclaving was 5.4 μm while after autoclaving the average size was 15.0 μm.

EXAMPLE 6

Embolization Composition

A 1 oz amber wide mouth bottle was rinsed with NanoPure water several times. The cap was rinsed with 70% isopropyl alcohol followed by NanoPure water and set aside. The bottle was filled with 15 mL preconditioned 1.1 mm zirconium silicate beads, covered with aluminum foil and depyrogenated for 8 hours at 240°C. All other glassware necessary to prepare surfactant, excipient or buffer solutions was depyrogenated. Any other remaining equipment was autoclaved. A 20 mL suspension in NanoPure water of 10% NC 12901 and 10% iohexol was prepared using solutions prepared by aseptic technique and filtered through sterile filters (ie. 0.2 micron Acrodisc® filter). The bottle was filled to capacity such that no air head space was present. The bottle was sealed with the above cleaned cap and roller milled for 24 hours. After milling was completed, the suspension was harvested into sterile (ie. rinsed and autoclaved) glass vials without further dilution and sealed with standard Teflon lined stoppers. The sealed vials were then autoclaved for 15 minutes at 121.1°C. Particle size, pH and osmolality were measured and recorded on extra samples prepared in parallel for testing.

EXAMPLE 7

Embolization Composition

Example 6 was repeated using NC 8883 in place of NC 12901.

EXAMPLES 8 AND 9

Examples 6 and 7 respectively were repeated using 5% iohexol in place of 10% iohexol.

The particle sizes for the compositions of Examples 6 to 9 were as follows:

Example	Dosage before Autoclaving	Particle size (μm) After Autoclaving
6	2.6	5.6
7	5.4	15.0
8	5.9	8.1
9	4.3	6.6

EXAMPLE 10

Embolus Formation in the Rabbit

0.1 mL of the composition of Example 6 was injected into the circumflex coronary artery of the rabbit to create emboli in the myocardium. After 10 minutes an X-ray CT image of the rabbit was recorded. This image, Figure 1 of the accompanying drawings, shows the embolus created.

EXAMPLE 11

The Formulation of Nanoparticles as Embolic Agents

A suspension of NC 70146 is prepared by adding 22.5 gm (22.5%, wt/vol %) of NC 70146 to a brown glass vial together with 4.5 gm (4.5%, wt/vol %) of biolpaque (NC 8851) and approximately 87 ml of water. Enough 1.1 mm zirconium silicate milling beads is added to fill the

glass jar halfway and the suspension is milled for three days at 150 rpm. At the end of this time, the particles are pipetted away from milling beads and sized at approximately 100 nm in average diameter using the Horiba 910a particle sizing instrument. After autoclaving, these beads are approximately 150 nm in average particle size.

Upon addition of this suspension to plasma or whole blood, the suspension will aggregate and gel, forming a clot within the plasma or blood stream and thus giving rise to an embolus-forming suspension.

EXAMPLE 12

6 to 10 micron particles of Hydroxyapatite prepared with Iohexol for Embolization

A 20 ml slurry of hydroxyapatite was prepared using 1.0g of hydroxyapatite and 7.6g of iohexol (solid) in 15.31 ml of water. This slurry was added to a 1 oz brown glass bottle along with 15 ml of 1.1 mm diameter zirconium silicate milling beads. The resulting slurry is 5% hydroxyapatite and 38% iohexol (wt/vol%). This slurry was rolled at approximately 100 rpm overnight. At the end of that time, the slurry had been transformed into a white, milky suspension with a pH = 7.28. Particle size was determined by light scattering using a Horiba 910a particle sizing instrument. After milling, the average particle size was determined to be 7.3 microns with a broad standard deviation of 5.4 microns. After autoclaving, the average particle size was determined to be 7.1 microns, again with a broad particle size distribution of 4.2 microns. As observed before with NC 12901, these large particles settle with time but are easily resuspended with gentle shaking.

These particles were examined in an acute pig model where 3.0 ml of suspension was administered directly into the renal artery by surgical cutdown on an anesthesized pig. Blood flow was then reestablished for 10 minutes before the kidney was imaged by conventional X-ray at 50 kV and 2 mamps. The X-ray clearly showed the complete kidney to be embolized by the agent. In addition, by comparison with a hydroxyapatite suspension without the iohexol added in the other kidney, it was further clear that the embolus traps the iohexol within the tissue. Thus, the drug delivery aspects of this embolic agent to the embolized tissue are confirmed (ie as iohexol can be delivered, so too can other "drugs").

Claims

1. A method of embolus therapy comprising administering into the vasculature of a perfused zone of tissue in a human or non-human animal subject a composition comprising particles of a size or formulation selected to generate emboli at a target site within said subject, characterised in that as said particles are used solid water-insoluble particles of a non-radioactive diagnostically effective compound or vesicles encapsulating a non-radioactive diagnostically effective compound or a solution thereof, and in that embolus location is detected by a diagnostic imaging technique.

2. A method as claimed in claim 1 wherein said target site is in a capillary.

3. The use of solid water-insoluble particles of a non-radioactive diagnostically effective compound or vesicles encapsulating a non-radioactive diagnostically effective compound or a solution thereof for the manufacture of an embolus generating pharmaceutical composition for use in embolus therapy.

4. A pharmaceutical composition comprising embolus forming contrast-effective particles together with a physiologically tolerable sterile liquid carrier medium, characterised in that said particles are solid water-insoluble particles of a non-radioactive diagnostically effective compound or vesicles encapsulating a non-radioactive diagnostically effective compound or a solution thereof.

Abstract

Method

The invention provides a method of embolus therapy comprising administering into the vasculature of a perfused zone of tissue in a human or non-human animal subject a composition comprising particles of a size or formulation selected to generate emboli at a target site within said subject, characterised in that as said particles are used solid water-insoluble particles of a non-radioactive diagnostically effective compound or vesicles encapsulating a non-radioactive diagnostically effective compound or a solution thereof, and in that embolus location is detected by a diagnostic imaging technique.